

# **UCLA**

## **UCLA Previously Published Works**

### **Title**

Actin filament assembly by bacterial factors VopL/F: Which end is up?

### **Permalink**

<https://escholarship.org/uc/item/8zg4v147>

### **Journal**

The Journal of cell biology, 216(5)

### **ISSN**

0021-9525

### **Authors**

Vizcarra, Christina L  
Quinlan, Margot E

### **Publication Date**

2017-05-01

### **DOI**

10.1083/jcb.201702165

Peer reviewed

# Actin filament assembly by bacterial factors VopL/F: Which end is up?

Christina L. Vizcarra<sup>1</sup> and Margot E. Quinlan<sup>2,3</sup>

<sup>1</sup>Department of Chemistry, Barnard College, New York, NY 10027

<sup>2</sup>Department of Chemistry and Biochemistry and <sup>3</sup>Molecular Biology Institute, University of California, Los Angeles, Los Angeles, CA 90095

Competing models have been proposed for actin filament nucleation by the bacterial proteins VopL/F. In this issue, Burke et al. (2017. *J. Cell Biol.* <https://doi.org/10.1083/jcb.201608104>) use direct observation to demonstrate that VopL/F bind the barbed and pointed ends of actin filaments but only nucleate new filaments from the pointed end.

Although the actin cytoskeleton can be arranged into many shapes and sizes, formation of new filaments is not favored. Cells use so-called nucleators of three major classes to stimulate actin assembly in a spatiotemporally controlled manner: the Arp2/3 complex, formins, and tandem WH2 domain nucleators (Dominguez, 2016). There is often a link between the mechanism of nucleation and the structures built. For example, the Arp2/3 complex binds to an existing filament to nucleate a new filament off of its side. Branched actin networks are mechanically suited to pushing and are often associated with dynamic membranes or the leading edge of a motile cell. In contrast, many structures built by formins, which can both accelerate filament growth and cross-link actin filaments, in addition to nucleating, are elongated bundles. We know much less about the structures built by tandem WH2 domain nucleators and their mechanisms of nucleation. Tandem WH2 domain nucleators are both eukaryotic (Spire and Cordon-bleu) and prokaryotic (from the bacteria *Vibrio*: VopF, VopL, and VopN; and the bacteria *Rickettsia*: Sca2). Evidence suggests that there are differences in how they function but also some common themes. For example, in most cases, nucleation by tandem WH2 domain proteins requires an additional domain, adjacent to the WH2 domains, and dimerization is commonly observed (Dominguez, 2016).

Actin filaments are polar, with a slow-growing “pointed” end and a fast-growing “barbed” end (Fig. 1 B). The majority of in vivo filament growth is attributed to elongation at the barbed end. There are outstanding questions about which end of a filament is bound by tandem WH2 domain nucleators. Originally, WH2 domains were described as actin monomer-binding domains. Given that WH2 domains bind to actin monomers at a site exposed at the barbed end (between subdomains 1 and 3; Fig. 1 A), one might reasonably suppose that tandem WH2 domain nucleators also associate with the barbed end of actin filaments.

Evidence for both barbed and pointed end binding has been presented for the bacterial nucleators *Vibrio parahaemolyticus* VopL and *Vibrio cholerae* VopF (VopL/F; Tam et al., 2007;

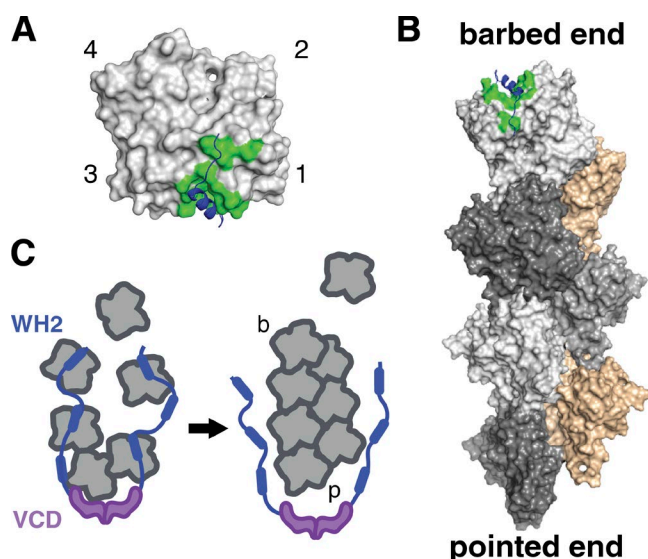
Namgoong et al., 2011; Pernier et al., 2013; Zahm et al., 2013). Two groups proposed that VopL nucleates actin filaments from the pointed end but only remains transiently associated with the new filament (Namgoong et al., 2011; Yu et al., 2011). Like other tandem WH2 nucleators, VopL/F depends on a series of WH2 domains (three) and an adjacent domain, which dimerizes (the VopL C-terminal domain [VCD]). Both in vitro biochemical assays and a structure containing the VCD dimer bound to the pointed end of three actin monomers in a filament-like conformation support the model (Zahm et al., 2013). In contrast, another group reported that VopF binds the barbed end of growing filaments and also severs filaments (Pernier et al., 2013). Given the similarity of VopL and VopF (32% sequence identity and 72% sequence similarity), one must question whether there is truly a mechanistic difference between these nucleators. Given these conflicting results, many questions remain. How do VopL/F nucleate? Do they remain associated with either end of a filament upon nucleation? If so, do they alter elongation? Do they bind either end of a filament independent of nucleation? In this issue, for the first time, Burke et al. provide single-molecule resolution analyses of bacterial tandem WH2 domain nucleators VopL/F to determine their mode of nucleation.

Burke et al. (2017) used single-molecule multicolor total internal reflection fluorescence microscopy to study nucleation by VopL/F side by side. They labeled purified proteins, including VopL/F, actin, and other actin binding proteins, to directly observe the nucleators and determine which end of the actin filament they bind. Burke et al. (2017) present evidence that the two nucleators function indistinguishably. They nucleate at the pointed end of a filament while remaining only briefly bound to that end, sometimes referred to as a “template” nucleation model (Fig. 1 C). In an effort to reconcile seemingly conflicting observations, the group performed a series of experiments under varying conditions, including (1) the presence or absence of preassembled filaments, (2) the presence or absence of excess actin monomers, and (3) the presence or absence of the actin monomer binding protein, profilin. These comparisons proved to be important, as has been observed for other tandem WH2 domain nucleators. Burke et al. (2017) found that VopL/F are pointed-end nucleators that fall off of a new filament after 1–2 min. They also show that VopL/F bind the ends of preassembled filaments only in the absence of free actin monomers. Under these conditions, VopL/F do not exhibit a preference for

© 2017 Vizcarra and Quinlan This article is distributed under the terms of an Attribution–Noncommercial–Share Alike–No Mirror Sites license for the first six months after the publication date (see <http://www.rupress.org/terms/>). After six months it is available under a Creative Commons License [Attribution–Noncommercial–Share Alike 4.0 International license, as described at <https://creativecommons.org/licenses/by-nc-sa/4.0/>].

Correspondence to Margot Quinlan: [margot@chem.ucla.edu](mailto:margot@chem.ucla.edu)





**Figure 1. WH2-actin interactions.** (A) Actin is shown in the standard orientation with the subdomains labeled. The WH2 domain (blue) binds between subdomains 1 and 3 of monomeric actin (highlighted in green; PDB 3M1F). (B) In structural models of the actin filament (PDB 2ZWH shown here), the WH2 binding site is accessible at the barbed end of the filament but not the pointed end. (C) Based on the results of Burke et al. (2017), VopL/F is a dimer that nucleates new filaments from the pointed end. Shortly thereafter, the filament is released.

barbed versus pointed ends and only remained associated with either end for about half a minute, suggesting that this association is distinct from the nucleating interaction. Experiments with profilin were consistent with these findings.

The implication is that monomer-binding versus filament-binding kinetics and thermodynamics cannot be ignored. For example, if a protein binds monomers rapidly, as one might expect in the case of a nucleator, the classical test of end binding by depolymerization of preformed filaments may be misleading because of the low levels of actin monomer present. Spire, which contains four WH2 domains, was reported to nucleate from the pointed end, albeit weakly (Quinlan et al., 2005), and to bind the barbed end of growing filaments and inhibit further growth (Bosch et al., 2007). These conflicting data regarding whether Spire binds the pointed or barbed end come from inhibition of depolymerization or polymerization assays, respectively, leading us to now favor the barbed end-binding model for Spire (Quinlan et al., 2005; Bosch et al., 2007). Further, severing assays must be considered with the same care. Although it is easier to preform filaments and then add a putative severing protein, it has been shown, at least in the case of Spire, that the presence of actin monomers has a strong impact on severing activity (Chen et al., 2012). In another case, severing experiments were performed with near stoichiometric amounts of VopF to actin, a condition that may not be physiologically relevant and could reflect sequestration as opposed to severing (Pernier et al., 2013).

Burke et al. (2017) nicely demonstrated the power of multicolor total internal reflection fluorescence with the addition of VopL/F to a mixture of filaments and monomers. More polymer was created both by elongation of the preexisting filaments and by nucleation of new filaments. This increase in actin assembly would be detected in a bulk assay. However, VopL/F were only associated with the new filaments, which would be

difficult to discern in either single-color imaging or bulk assays. Because the large majority of experiments with VopF were performed in bulk, it is possible that the reactions were more complicated than assumed when interpreting the data, leading to some of the discrepancies.

Although Burke et al. (2017) resolve the role of actin monomers in controlling filament binding, discrepancies between the VopL/F mechanistic models remain. For instance, it is difficult to reinterpret the data demonstrating that VopF competes with capping protein, a well-characterized barbed end capper (Pernier et al., 2013). Further, protection from capping protein was accompanied by barbed end growth, indicating a processive association between VopF and the filament barbed end. No such processive barbed end association was observed by Burke et al. (2017). Instead they observed that VopL/F association with the barbed end almost completely arrests filament growth. In addition, when VopL/F and capping protein are present at a 1:10 ratio, the two proteins are observed at opposite ends of the filament from capping protein. However, Namgoong et al. (2011) described rare cases of processive association for quantum-dot immobilized VopL (~18%). A subset of these (~7%) were bound at or near the barbed end and grew more than twice as quickly as other filaments. It is also difficult to reconcile a template nucleation model with localization studies in mammalian cells that show VopF present at filopodial tips, which are rich in barbed ends (Tam et al., 2007). In the spirit of the study by Burke et al. (2017), perhaps a systematic study of VopL and VopF in cells using super-resolution microscopy could further resolve filament interaction models.

The study by Burke et al. (2017) provides compelling new evidence for the template nucleation model (Fig. 1 C), agreeing nicely with a structure that indicates an affinity of the VCD with the pointed end of a filament nucleus (Zahm et al., 2013). That said, questions remain, including whether the template nucleation model applies to other tandem WH2 domain nucleators. A crystal structure of the WH2 domains and dimerization domain of VopL/F bound to actin could reveal how the full nucleating apparatus of VopL/F engages actin. By combining biochemical studies, both bulk and at the single-molecule level, with structural work, our understanding of WH2-based actin nucleators is advancing.

## Acknowledgments

C.L. Vizcarra is funded by the generous support of Barnard College and the Sally Chapman Fund. M.E. Quinlan is supported by National Institutes of Health grant R01GM096133 and March of Dimes Foundation grant 1-FY15-290.

The authors declare no competing financial interests.

## References

- Bosch, M., K.H. Le, B. Bugyi, J.J. Correia, L. Renault, and M.F. Carlier. 2007. Analysis of the function of Spire in actin assembly and its synergy with formin and profilin. *Mol. Cell.* 28:555–568. <http://dx.doi.org/10.1016/j.molcel.2007.09.018>
- Burke, T.A., A.J. Harker, R. Dominguez, and D.R. Kovar. 2017. The bacterial virulence factors VopL and VopF nucleate actin from the pointed end. *J. Cell Biol.* <http://dx.doi.org/10.1083/jcb.201608104>
- Chen, C.K., M.R. Sawaya, M.L. Phillips, E. Reisler, and M.E. Quinlan. 2012. Multiple forms of Spire-actin complexes and their functional

- consequences. *J. Biol. Chem.* 287:10684–10692. <http://dx.doi.org/10.1074/jbc.M111.317792>
- Dominguez, R. 2016. The WH2 Domain and Actin Nucleation: Necessary but Insufficient. *Trends Biochem. Sci.* 41:478–490. <http://dx.doi.org/10.1016/j.tibs.2016.03.004>
- Namgoong, S., M. Boczkowska, M.J. Glista, J.D. Winkelman, G. Rebowski, D.R. Kovar, and R. Dominguez. 2011. Mechanism of actin filament nucleation by *Vibrio* VopL and implications for tandem W domain nucleation. *Nat. Struct. Mol. Biol.* 18:1060–1067. <http://dx.doi.org/10.1038/nsmb.2109>
- Pernier, J., J. Orban, B.S. Avvaru, A. Jégou, G. Romet-Lemonne, B. Guichard, and M.-F. Carlier. 2013. Dimeric WH2 domains in *Vibrio* VopF promote actin filament barbed-end uncapping and assisted elongation. *Nat. Struct. Mol. Biol.* 20:1069–1076. <http://dx.doi.org/10.1038/nsmb.2639>
- Quinlan, M.E., J.E. Heuser, E. Kerkhoff, and R.D. Mullins. 2005. *Drosophila* Spire is an actin nucleation factor. *Nature*. 433:382–388. <http://dx.doi.org/10.1038/nature03241>
- Tam, V.C., D. Serruto, M. Dziejman, W. Briher, and J.J. Mekalanos. 2007. A type III secretion system in *Vibrio cholerae* translocates a formin/spire hybrid-like actin nucleator to promote intestinal colonization. *Cell Host Microbe*. 1:95–107. <http://dx.doi.org/10.1016/j.chom.2007.03.005>
- Yu, B., H.C. Cheng, C.A. Brautigam, D.R. Tomchick, and M.K. Rosen. 2011. Mechanism of actin filament nucleation by the bacterial effector VopL. *Nat. Struct. Mol. Biol.* 18:1068–1074. <http://dx.doi.org/10.1038/nsmb.2110>
- Zahm, J.A., S.B. Padrick, Z. Chen, C.W. Pak, A.A. Yunus, L. Henry, D.R. Tomchick, Z. Chen, and M.K. Rosen. 2013. The bacterial effector VopL organizes actin into filament-like structures. *Cell*. 155:423–434. <http://dx.doi.org/10.1016/j.cell.2013.09.019>